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CONFORMATION OF DERIVATIVES OF BOVINE α-LACTALBUMIN OBTAINED BY CYANOGEN BROMIDE ATTACK

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Received 16 July 1971

1. Introduction

The conformational study of peptide fragments, comprising partial amino acid sequences of proteins, is a well established approach to the understanding of the role played by short-range and long-range interactions in the mode of folding of proteins. Furthermore, the study of the properties of isolated segments of protein molecules, particularly those involving the amino terminal regions can provide information on the folding of proteins *in vivo* [1–14]. Actually, studies of the rate of hemoglobin chain synthesis in a reticulocyte ribosome system have been interpreted to suggest that folding is taking place during chain synthesis [15]. In addition, recent studies on the *in vitro* renaturation of proteins [16] show that the rate of regain of gross conformational structure *in vitro* is faster than the rate of polypeptide chain synthesis *in vivo*. The initial folding process occurs in less than a minute whereas the synthesis of a polypeptide chain of 35,000 M.W. requires at least 5 min [17]. This again suggests that folding will occur during the biosynthesis. Now the question arises: is the folding which takes place before completion of the chain the proper, i.e. native, folding? Or, because of the subsequent influence of residues nearer the carboxyl terminus, the initial structure will be distinct from that produced when the synthesis of the molecule is complete? To obtain further insights into this problem we shall discuss here the near and far-ultraviolet circular dichroism spectra of unmodified and cyanogen bromide-cleaved α-lactalbumin (CB-Lac). CB-Lac is a special type of modified protein, the two peptide fragments of which are held together in the unreduced molecule by four disulfide bonds.

Moreover, the two peptides obtained by reduction of CB-Lac, comprising respectively the sequence 1–90 (CB-1) and 91–123 (CB-2), were also studied by circular dichroism.

2. Experimental procedure

Bovine α-lactalbumin was purchased from Pentex Inc., Kankakee, Ill. and purified as elsewhere reported [18]. Ultrapure guanidinium chloride was obtained from Mann Research Laboratories Inc., New York, NY 2-mercaptoethanol and iodoacetic acid were products of Fluka, Basel (Switzerland).

Cyanogen bromide was purchased from Schuchardt, Munchen (Germany). BC-Lac was prepared according to Brew and Hill [19]. Amino acid analysis of the product was in good agreement with the expected values; in particular, no methionine was present and peaks, corresponding to homoserine and homoserine lactone, appeared. BC-Lac was reduced and carboxymethylated by 2-mercaptoethanol and iodoacetic acid in 6 M guanidinium chloride, pH 8.6, under the same conditions used by Brew et al. [20] for α-lactalbumin. The resulting peptides were isolated by column chromatography on Sephadex G-100 equilibrated with 50% acetic acid (fig. 1). Amino acid analysis revealed the disappearance of methionine and the presence of homoserine and homoserine lactone in CB-1 and S-carboxymethyl cysteine in both peptides in the expected ratio. Values for the remaining amino acid residues were in agreement with those calculated from the sequences. Circular dichroism data were obtained with a Cary Model 60 recording spectropolarimeter equipped with a 6001 dichroism

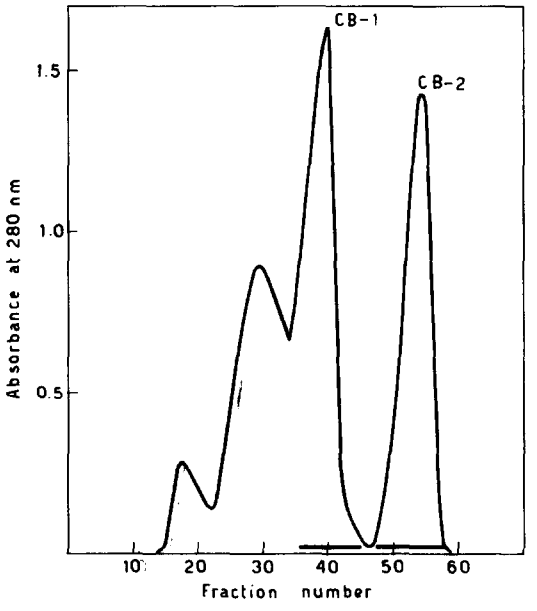

Fig. 1. Separation on Sephadex G-100 (5 × 100 cm) of the products formed on reduction of CB-Lac. Eluent 50% (v/v) acetic acid.

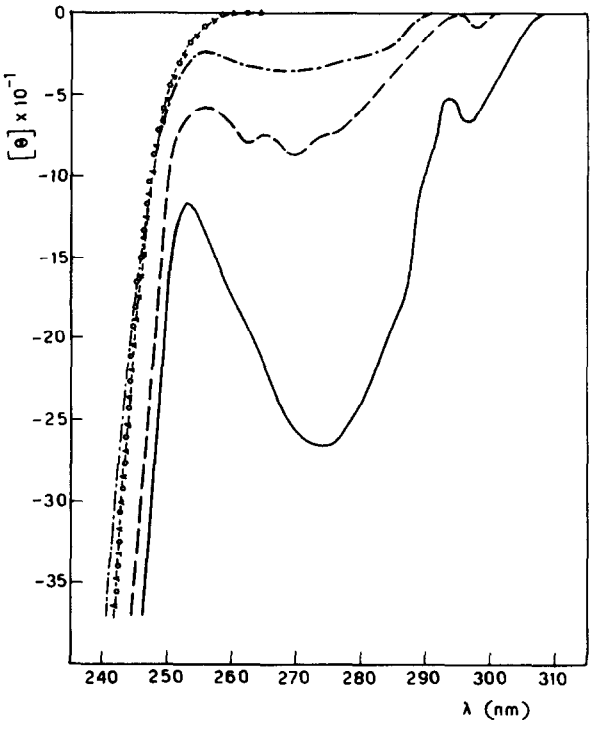

Fig. 2. Near-ultraviolet circular dichroism spectra in 0.025 M. Tris-chloride buffer, pH 7.3: (——) α-lactalbumin; (- - - -) CB-Lac; (–○–○–) CB-1; (–△–△–) CB-2; (–.–.–) α-lactalbumin in 6 M guanidinium chloride.

accessory. All measurements were made at ambient temperature. Cylindrical quartz cells were used with 10, 1.0, 0.1, and 0.05 cm optical path. The usual instrumental precautions were taken to avoid artifacts. Samples of peptides and proteins were examined at concentrations ranging from 0.4 to 0.8 mg/ml. The data are expressed in terms of $[\theta]_\lambda$, the mean residue molecular ellipticity, in units of deg cm^2 $dmole^{-1}$.

Acid hydrolysis of the products was performed for 22 hr in evacuated, sealed tubes at 100° in 6 M hydrochloric acid. The dried hydrolysate was subjected to amino acid analysis on a Carlo Erba 3A27 automatic analyzer according to the procedure of Spackman et al. [21].

3. Results and discussion

Fig. 2 contains the circular dichroism spectra of CB-Lac, CB-1, and CB-2 in aqueous solution, pH 7.3, compared to the native and the guanidinium chloride-denaturated protein. The circular dichroism spectra of CB-Lac in the aromatic region (fig. 2) show a general lowering of ellipticity with respect to the unmodified protein. A similar effect, albeit more pronounced, is observed in the presence of guanidinium chloride.

In the far-ultraviolet region (fig. 3) the shape of the spectrum changes significantly in comparison to the native protein. The minimum at 208 nm becomes more prominent and increases in ellipticity while the curve becomes steeper at longer wavelengths. It should be noted that the observed spectral variation is not that expected for an increase of unordered structure: actually, there is little resemblance with the spectrum of guanidinium chloride denatured α-lactalbumin characterized by the dichroic pattern which is assumed to be typical of essentially disordered proteins without residual non-covalent structure [22]. On the contrary, the striking resemblance with acid- and alkaline denatured α-lactalbumin is evident, and it has been suggested that the conformational changes

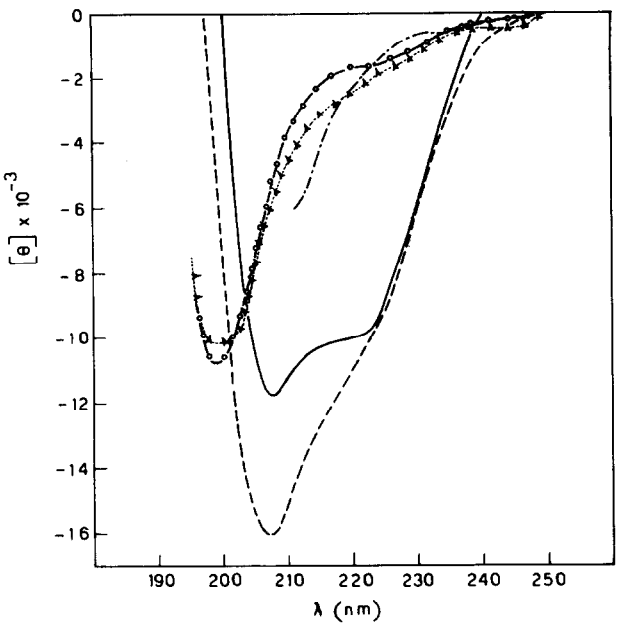

Fig. 3. Far-ultraviolet circular dichroism spectra. Symbols as in fig. 2.

result in an apparent increase in α-helix content and a decrease in β-structure [23]. Thus, it seems that the conversion of the methionine 90 to homoserine lactone with concomitant peptide chain cleavage, obviously associated with local distorsions very close to the point of chain disruption, might also exert a more profound effect on the shape of the molecule.

The near-ultraviolet spectra of CB-1 and CB-2 show no discernible dichroism above 260 nm, while in the far-ultraviolet region the most prominent feature for both peptides is the negative band centered at about 199 nm clearly associated with the amide $\pi-\pi^*$ transition of unordered polypeptides. All these data strongly suggest that both CB-1 and CB-2 are essentially structureless in aqueous solution. This means that the conformation of the peptides in the free state and in the intact protein are different, as the rigid structure of the native protein is reflected by quite different dichroic spectra in the near- and ultraviolet. Therefore, when covalently linked*, the two peptides are interacting by a set of long-range interactions which stabilize the whole structure.

Finally, as CB-1 constitutes the *N*-terminal part of α-lactalbumin, its conformational properties reserve further comments in connection with the folding during biosynthesis. Actually, one may infer, from the present data, that the native conformation of α-lactalbumin cannot be formed during biosynthesis until the polypeptide chain has been extended beyond residue 90, because of the importance of contribution to long-range interactions of the yet-unsynthesized portion. Thus, if, as mentioned folding occurs before synthesis is completed, the initial folding will be uncorrect and the protein will quickly equilibrate to the most stable conformation. This thermodynamically-controlled folding seems a rather general process [2, 6, 9–14, 16], albeit some examples of folding under kinetic control are also available [16].

* An attempt was made to restore the non-covalent interactions between CB-1 and CB-2 in absence of the covalent linkage 90–91 and the disulfide bonds. However, no evidence of interaction was found, as the equimolar mixture of CB-1 and CB-2 gave a circular dichroism curve comparable, within experimental error, to the sum of the curves of the individual peptides.

References

[1] M.J. Crumpton and P.A. Small, J. Mol. Biol. 26 (1967) 143.
[2] A. Scatturin, A.M. Tamburro, R. Rocchi and E. Scoffone, Chem. Commun. (1967) 1273.
[3] W.A. Klee, Biochemistry 7 (1968) 2731.
[4] E.R. Simons and E.R. Blout, J. Biol. Chem. 243 (1968) 218.
[5] M.J. Crumpton, Biochem. J. 108 (1968) 18 P.
[6] R.M. Epand and H.A. Scheraga, Biochemistry 7 (1968) 2864.
[7] J.E. Brown and W.A Klee, Biochemistry 8 (1969) 2876.
[8] A. Bodanszky, M.A. Ondetti, V. Mutt and M. Bodanszky, J. Am. Chem. Soc. 91 (1969) 944.
[9] H. Taniuchi and C.B. Anfinsen, J. Biol. Chem. 244 (1969) 3864.
[10] A.M. Tamburro, E. Boccu and L. Celotti, Intern. J. Protein Res. 2 (1970) 157.
[11] H. Taniuchi, J. Biol. Chem. 245 (1970) 5459.
[12] R.P. Singhal and M.Z. Atassi, Biochemistry 9 (1970) 4252.
[13] E. Scoffone, F. Marchiori, R. Rocchi, A. Scatturin and A.M. Tamburro, Proceedings of the Tenth European Peptide Symposium, ed. E. Scoffone (North-Holland, Amsterdam, 1971) p. 233.
[14] C.A. Benassi, R. Ferroni, M. Guarneri, A. Guggi, A.M. Tamburro, R. Tomatis and R. Rocchi, FEBS Letters 14 (1971) 346.

[15] R.M. Winslow and V.M. Ingram, J. Biol. Chem. 241 (1966) 1144.
[16] J.W. Teipel and D.E. Koshland, Jr., Biochemistry 10 (1971) 798.
[17] T. Hunt, F. Hunter and A. Munro, J. Mol. Biol. 43 (1969) 123.
[18] A.M. Tamburro, G. Jori, G. Vidali, A. Scatturin and G. Saccomani, in preparation.
[19] K. Brew and R.L. Hill, J. Biol. Chem. 245 (1970) 4559.
[20] K. Brew, F.J. Castellino, T.C. Vanaman and R.L. Hill, J. Biol. Chem. 245 (1970) 4570.
[21] D.H. Spackman, W.H. Stein and S. Moore, Anal. Chem. 30 (1958) 1190.
[22] C. Tanford, Advan. Protein Chem. 23 (1968) 121.
[23] F.M. Robbins and L.G. Holmes, Biochim. Biophys. Acta 221 (1970) 234.